

hybridization on microarray. The assay was evaluated using 212 DNA samples isolated from clinical specimens containing STDs agents. Real-time PCR, target sequencing and partial conventional drug susceptibility testing were used as reference tests.

Results: A developed method provides identification of 17 different obligate and conditional pathogens including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*, *Mycoplasma genitalium*, *Atopobium vaginae*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Mobiluncus mulieris*, *Mycoplasma hominis*, *Staphylococcus epidermidis*, *Streptococcus anginosus*, *Trichomonas vaginalis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* with coincident analysis of 49 genetic determinants of resistance to macrolides, aminoglycosides, tetracyclines, aminocyclites, quinolones and nitroimidazoles. Sensitivity and specificity of the assay exceeded 95% for identification of STDs agents, and were 80–92% for detection of resistance to different antimicrobial drugs.

Conclusion: The proposed multiplex assay is a useful tool for selection of personalized treatment of STDs providing rapid and accurate identification of pathogens in clinical samples with simultaneous determination of resistance markers to a series of antimicrobial drugs. This work was financed by subsidy #14.607.21.0065 (RFMEFI60714X0065) from the Ministry of Science and Education of Russian Federation.

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Temperature and oxidative stress as triggers for virulence gene expression in pathogenic *Leptospira* spp.

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Background: Leptospirosis is a zoonanthroponosis, widely distributed throughout the world and aetiologically caused by pathogenic bacteria belonging to the genus, *Leptospira*. Environmental signals such shifts from environmental (30 °C) to physiological (37 °C) temperatures or increases in oxidative stress can trigger response regulatory modes of the organism during the infection process. These responses might be mediated by upregulation, attenuation or silencing of genes whose expression is secondary to the organism's survival. This study sought to determine the effect of temperature and oxidative stress on virulence associated genes in highly-passaged *L.borgpetersenii* Jules and *L. interrogans* Portlandvere.

Methods & Materials: Bacteria were grown in EMJH at 30 °C, 37 °C or at 30 °C before being transferred to 37 °C. A total of 16 virulence-associated genes (*lipL45*, *tlyC*, *lsa24*, *fliY*, *lflh*, *ligB*, *mce*, *lipL36*, *loa22*, *invA*, *ompL1*, *spH2*, *lipL32*, *hap1*, *lsa21/lenA*, and *lipL41*) were assessed using endpoint RT-PCR. To assess oxidative stress, bacteria were exposed to H₂O₂ for 30 and 60 min with or without the temperature stress.

Results: Transcriptional expression of virulence associated genes in *L. interrogans* Portlandvere (19%) was significantly lower

lated following elevated temperature or temperature shift included *loa22* (in both), *hap1* and *lipL32* in Portlandvere and *invA* and *lsa21* in Jules. Genes expressed during oxidative stress included *loa22*, *lipL32* and *lipL41* in both serovars, and *fliY* and *lsa21* exclusively in Portlandvere.

Conclusion: While it is clear that expression of many virulence genes in highly passaged strains of *Leptospira* are attenuated or lost, the differences in gene expression between *L. interrogans* Portlandvere and *L. borgpetersenii* Jules may be attributed to the transmission cycle of the bacteria and/or regulation by other contributing factors. Overall, serovar Jules retained more potential for causing infection even though highly passaged.

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Prevalence of virulence determinants among HA-MRSA and CA-MRSA isolates and pathogenicity testing using caenorhabditis elegans model

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has established itself as a major human pathogen causing both hospital and community acquired infections, especially due to its ability to express a myriad of virulence factors. There is paucity in data regarding the prevalence of virulence factors among MRSA, its role in pathogenesis and associated severity of infections from our part of the country. Hence, the present study has been designed to use *C. elegans*, a simple nematode model for pathogenicity testing and to demonstrate the virulence potential of HA & CA-MRSA.

Methods & Materials: A total of 100 *S. aureus* clinical isolates from community and hospital settings were included for the study. Methicillin resistance was screened using cefoxitin disc (30 µg) and confirmed by a rapid triplex PCR for *mecA*, *femA*, *pvl* genes. Multiplex PCR was done to detect leukocidins (*lukD*, *lukE*, *lukM*), hemolysins (*hla*, *hly*, *hld*, *hlg*) and 14 enterotoxins (*sea*, *see*, *seg*, *seo*). Nematode killing assay was performed by exposing stage-synchronized *C. elegans* L4-larvae to four representative HA-MRSA and CA-MRSA strains and phase-contrast microscopy was done to observe pathological effects.

Results: Of the 100 *S. aureus* isolates, 39 and 61 isolates were from hospital and community settings of which 64% were MRSA. 33/61 isolates (54%) from community settings were CA-MRSA and 31/39 (79%) isolates from hospitalised patients were HA-MRSA. Among the leukocidins tested, prevalence of *pvl* was highest (38%) followed by *lukD* and *lukE* in 32% of isolates. All isolates were

